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THE EFFECT OF SPLENECTOMY AND  
BLOCKADE ON THE PROTECTIVE  
TITER OF ANTISERUM AGAINST  
TRYPANOSOMA EQUIPERDUM

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION  
OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE  
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY

1936

By  
LUDWIG ROLAND KUHN

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# THE EFFECT OF SPLENECTOMY AND BLOCKADE ON THE PROTECTIVE TITER OF ANTISERUM AGAINST TRYPANOSOMA EQUIPERDUM

L. R. KUHN

From the Department of Bacteriology and Parasitology, University of Chicago, Chicago,  
and the Department of Bacteriology and Preventive Medicine,  
University of Texas Medical Department, Galveston

This publication concerns the passive protective titers of sheep anti-*Trypanosoma equiperdum* serum in normal, splenectomized, and splenectomized and blockaded mice. It contains an elaboration of a preliminary report by Taliaferro and Kuhn<sup>1</sup> and includes a detailed description (fig. 1) of one experiment recorded by them.

The literature dealing with the passive resistance of blockaded animals was reviewed recently by Taliaferro,<sup>2</sup> who found that blockaded rats require more rat trypanocidal serum to protect them against infection with *T. lewisi* than normal rats, but that blockaded rats can be effectively immunized passively with ablasic anti-*T. lewisi* serum. Inasmuch as he could recognize no effect of blockade upon the rate of disappearance of sensitized trypanosomes from the peripheral blood, he considered that the effect of blockade upon the protective trypanocidal titer might be ascribed to interference with the union in vivo of trypanosomes and antibody in optimal proportions. Since agglutination of the sensitized trypanosomes prevents a satisfactory study of their rate of disappearance, however, he concluded that the ultimate explanation of the effect of blockade upon the protective trypanocidal activity of the serum awaits direct examination of the tissues.

In the work here presented, a pathogenic trypanosome and specific sheep serum were used to study the protective trypanocidal property of the serum in normal, in splenectomized and in splenectomized and blockaded mice. Although no attempt was made to exclude ablasic activity all evidence indicates that antisera for pathogenic trypanosomes are purely trypanocidal without reproduction-inhibiting properties (see review in Taliaferro<sup>3</sup>).

## MATERIALS AND METHODS

The strain of *T. equiperdum* used throughout this investigation was maintained in mice and was transferred by intraperitoneal injections of infected blood. Such passage strains are antigenically stable (Taliaferro<sup>3</sup>).

Specific protective serum was obtained from sheep which had been infected for at least two weeks with a passage strain of *T. equiperdum*. In the protection tests in fig. 1 (exper. 1) and in table 2 (experiments 1 to 9), doses of the immune serum varying from 0.1 cc. or 0.2 cc. up to 0.6,

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1. *J. Parasitol.* **19**: 158, 1932.

2. *J. Infect. Dis.* **62**: 98, 1938.

3. *The Immunology of Parasitic Infections*, New York Century, 1929, p. 414.

0.8 or 1.0 cc. by 0.1 cc. steps were given to a series of normal and splenectomized mice at the time of the trypanosome injection (i.e., 5 to 10 normal and 5 to 10 splenectomized mice were used to titrate the immune serum protectively) and from 0.1 cc. or 0.2 cc. (with the exception of experiment 7, where the smallest dose was 0.3 cc.) to 2.0 cc. were given to a series of splenectomized-blockaded mice (i.e., 19 or 20 splenectomized-blockaded mice were used to titrate the immune serum protectively). In general, the doses of 1.8, 1.9 and 2.0 cc. were too much for the mice, and the mice died within 2 to 3 days, that is, before trypanosomes appeared in their blood (see fig. 1), but, fortunately, those receiving other doses lived and the titration could be ade-

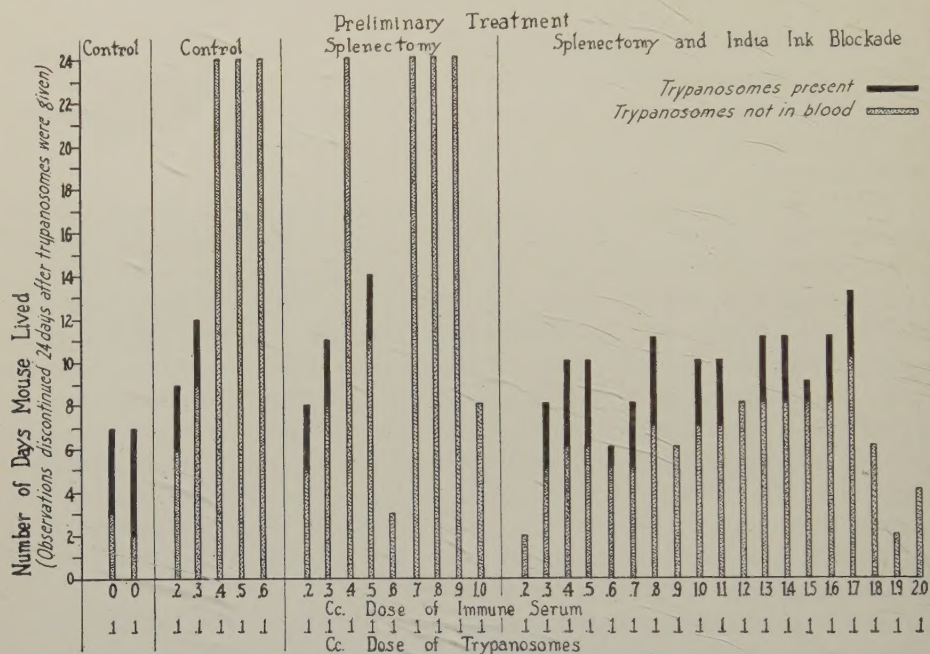


Fig. 1 (exper. 1).—The effect of splenectomy plus blockade with India ink on the protective titer of sheep anti-*T. equiperdum* serum as indicated by the presence or absence of trypanosome infection in five normal, nine splenectomized and nineteen splenectomized and blockaded mice given a standard dose of 2,500 to 3,000 trypanosomes and different doses of serum, and in two normal mice given only trypanosomes. (From data of Taliaferro and Kuhn.<sup>1</sup>)

quately ascertained. For these tests, each class of mice (i.e., normal or treated) was arranged in order by weight and to the larger mice were given the larger doses of serum. On the whole, the mice did not vary more than 6 grams in any one experiment. Some lost weight during the process of splenectomy and blockade. All doses of serum were given, intraperitoneally, per 20 grams mouse body weight at the time of the serum injection. Immediately after, 0.1 cc. of a suspension of trypanosomes in mouse blood, diluted in cold Ringer's dextrose solution, was injected subcutaneously. The number of trypanosomes was regulated after counting samples of the suspension on a Levy-Hauser haemocytometer.

The presence or absence of trypanosome infection was determined daily for 2 weeks and twice weekly for 2 weeks thereafter by examining fresh blood from the tail with a 4 mm. objective. Since trypanosomes are found 2 to 4 days in the blood of mice before they die, and since the trypanosomes increase in number without interruption by crises, daily examination of the blood precludes failure to diagnose infection. The number of days various mice survived after receiving trypanosomes is represented in fig. 1 by correspondingly long shaded or shaded and solid lines which also signify absence (shaded portion) or presence (solid portion) of trypanosome infection. These data, together with similar data, collected from 8 more experiments,



are summarized in table 1 by simply giving the critical dose of serum which was the least amount in a given series that protected. Such an amount is termed the protective titer of the particular serum for the type of mouse indicated. Thus, in table 1, experiment 2, a dose of 0.4 cc. of immune serum or larger protected a normal mouse against an infection of 2500 to 3000 trypanosomes, whereas a dose of 0.3 cc. or lower did not protect a normal mouse from a similar infection; a dose of 0.3 cc. or larger of the same serum protected a splenectomized mouse against the same number of trypanosomes, whereas a dose of 0.3 cc. or lower did not protect a splenectomized mouse from a similar infection; and finally, a dose of 1.0 cc. or larger of the same serum protected a splenectomized and blockaded mouse against a similar infection, whereas a dose of 0.9 cc. or lower did not protect a splenectomized and blockaded mouse from a similar infection.

TABLE 1.—*The Passive Protective Titers of Sheep Anti-T. Equiperdum Serum in Normal, in Splenectomized and in Splenectomized and Blockaded Mice, as Indicated by the Least Amount (in cc.) of Serum giving Protection Against Trypanosome Infection in Nine Infections*

|                                   | 1                          | 2                          | 3             | Experiment number <sup>1</sup> |                    |                  | 7                  | 8             | 9             |
|-----------------------------------|----------------------------|----------------------------|---------------|--------------------------------|--------------------|------------------|--------------------|---------------|---------------|
|                                   |                            |                            |               | 4                              | 5                  | 6                |                    |               |               |
| Normal Mice                       | 0.4                        | 0.4                        | 0.5           | 0.5                            | 0.5                | 0.4              | 0.6                | 0.3           | 0.25          |
| Splenectomized mice               | 0.6 <sup>2</sup>           | 0.3                        | 0.5           | 0.5                            | 0.5                | 0.6              | 0.5                | 0.3           |               |
|                                   |                            |                            |               | Blockading procedure:          |                    |                  |                    |               |               |
|                                   |                            |                            |               | Normal sheep serum             | Normal sheep serum | Normal dog serum | Immune sheep serum |               |               |
| Splenectomized and blockaded mice | India ink 1.8 <sup>3</sup> | India ink 1.0 <sup>4</sup> | India ink 1.4 | 1.6                            | 1.5                | 1.4              | 0.3 <sup>5</sup>   | India ink 0.5 | India ink 0.5 |

<sup>1</sup> Immediately after the dose of immune serum was given intraperitoneally, a standard dose of 2,500 to 3,000 trypanosomes in 0.1 cc. was given subcutaneously in experiments 1 through 7, and of 1,200 to 1,600 trypanosomes in experiments 8 and 9.

<sup>2</sup> All doses above 0.5 cc. protected, but the mouse receiving 0.4 cc. also was protected (see fig. 1).

<sup>3</sup> No dose protected through 1.7 cc. and mice receiving larger doses died before protection could be determined.

<sup>4</sup> 1.0 cc. of serum was the greatest amount given. No dose protected through 0.9 cc. and the mouse receiving 1.0 cc. died before protection could be determined.

<sup>5</sup> 0.3 cc. of serum was the least amount given.

Sensitized trypanosomes were prepared in the following manner: 2 heavily infected mice were bled into 40 cc. of cold Ringer's dextrose solution containing 1% sodium citrate. After the suspension was centrifuged slowly for 10 minutes, the supernatant was removed and centrifuged rapidly for 30 minutes. Then the second supernatant was discarded, and to the trypanosomes which were concentrated at the bottom of the tube was added inactivated serum from an infected sheep (4 parts of serum to 1 part of trypanosomes). This suspension was kept in an ice bath for one hour, during which it was frequently shaken and 5 cc. of Ringer's dextrose solution was added every 15 minutes. It was then centrifuged at high speed for 3 15-minute periods after each of which the supernatant was replaced with cold Ringer's dextrose solution. After the third centrifugation the number of trypanosomes was determined, and the suspension was injected.

In tables 2 and 3, experiments 10 to 16, the day of death is indicated by a number preceded by the letter D, and the day that trypanosome infection was first detected is shown by a number preceded by a positive sign (+). Absence of trypanosome infection is indicated by a zero (0).

Spleens were removed from mice under ether anesthesia after ligating the vessels with silk thread. In inducing blockade, mice were splenectomized and then injected on each of the 6 succeeding days, either with 7% India ink (except in experiments 13–16, vide infra) in 0.85% NaCl or with whole serum, 0.5 cc. intraperitoneally and 0.5 cc. intravenously. The protection test was done the day after the sixth blockading injection.

#### THE PROTECTIVE TITERS OF SHEEP ANTI-T. EQUIPERDUM SERUM IN NORMAL, SPLENECTOMIZED AND SPLENECTOMIZED AND BLOCKADED MICE

Specific sheep serum, in an intraperitoneal dose of 0.4 to 0.6 cc., usually protected normal mice against infection when 2,500 to 3,000 trypanosomes were injected in 0.1 cc. subcutaneously. Considerably more serum was re-

TABLE 2 (expers. 10-12).—*The Resistance of Normal and Splenectomized-Blockaded<sup>1</sup> Mice to Specifically Sensitized Trypanosomes given Subcutaneously or Intravenously*

| Experiment number                                                       |       |        |       |        |        |       |       |       |        |        |       |      |      |       |       |
|-------------------------------------------------------------------------|-------|--------|-------|--------|--------|-------|-------|-------|--------|--------|-------|------|------|-------|-------|
| 10                                                                      |       |        |       | 11     |        |       |       |       | 12     |        |       |      |      |       |       |
| cc. sensitized trypanosome suspension given: <sup>2</sup>               |       |        |       |        |        |       |       |       |        |        |       |      |      |       |       |
| 0.10                                                                    | 0.08  | 0.05   | 0.10  | 0.08   | 0.05   | 0.025 | 0.010 | 0.10  | 0.05   | 0.025  | 0.010 | 0.10 | 0.05 | 0.025 | 0.010 |
| Infection in normal mice:                                               |       |        |       |        |        |       |       |       |        |        |       |      |      |       |       |
| +7D10                                                                   | +8D11 | +11D13 | +6D9  | +9D12  | 0      | 0     | 0     | +8D12 | +12D15 | 0      | 0     |      |      |       |       |
| +6D10                                                                   | +7D11 | 0      | +8D12 | +8D11  | 0      | 0     | 0     | +8D11 | +12D15 | 0      | 0     |      |      |       |       |
| +6D9                                                                    | +8D12 | 0D8    | 0D6   | 11+D13 | +12D14 | 0     | 0D14  | +7D10 | 0      | +12D16 | 0     |      |      |       |       |
| +12D15                                                                  |       |        |       |        |        |       |       |       |        |        |       |      |      |       |       |
| Infection in splenectomized mice blockaded with India ink: <sup>1</sup> |       |        |       |        |        |       |       |       |        |        |       |      |      |       |       |
| +6D10                                                                   | +8D11 | 0      | +7D11 | +8D12  | 0      | 0     | 0     | +6D10 | +12D14 | 0      | 0     |      |      |       |       |
| +6D10                                                                   | +5D10 | 0D3    | +7D10 | 0D12   | +12D14 | 0     | 0     | +7D10 | +13D16 | 0      | 0     |      |      |       |       |
| +6D10                                                                   | +7D10 | +12D16 | +6D10 | +10D13 | +11D14 | 0D5   | 0     | +9D12 | 0D7    | 0      | 0D4   | 0    |      |       |       |
| 0                                                                       |       |        |       |        |        |       |       |       |        |        |       |      |      |       |       |

<sup>1</sup> For blockading procedure with India ink, see text.<sup>2</sup> 2,000 trypanosomes per 0.1 cc. given subcutaneously in experiments 10 and 11 and 1,000 trypanosomes per 0.1 cc. given intravenously in experiment 12.

+ = trypanosome infection and the number after it indicates the day after injection on which the trypanosomes were first seen; D = died and the number after it indicates the day after injection on which death occurred; 0 = no trypanosome infection.

TABLE 3 (expers. 13-16).—*The Resistance of Normal and Splenectomized-Blockaded<sup>1</sup> Mice to Specifically Sensitized Trypanosomes given Intravenously in Doses of 1,000 Trypanosomes per 0.1 cc.*

|        |                                                                         | Experiment number                            |       |        |       |        |      |
|--------|-------------------------------------------------------------------------|----------------------------------------------|-------|--------|-------|--------|------|
| 13     |                                                                         | 14                                           |       |        |       | 16     |      |
|        |                                                                         | cc. sensitized trypanosome suspension given: |       |        |       |        |      |
| 0.10   | 0.10                                                                    | 0.05                                         | 0.10  | 0.05   | 0.10  | 0.05   | 0.05 |
|        |                                                                         | Infection in normal mice:                    |       |        |       |        |      |
| +11D15 | +6D9                                                                    | +11D14                                       | +6D9  | +10D13 | +7D10 | +12D14 |      |
| +8D11  | +8D12                                                                   | +12D14                                       | +7D11 | 0      | +7D10 | 0      |      |
| 0      | +8D11                                                                   | 0                                            | +9D12 | 0      | +8D12 | 0      |      |
| 0      | 0                                                                       | 0                                            | +9D12 | 0D4    | +8D10 | 0      |      |
|        | Infection in splenectomized mice blockaded with India ink: <sup>1</sup> |                                              |       |        |       |        |      |
| +4D8   | +7D10                                                                   | +11D14                                       | +6D9  | +12D14 | +6D8  | 0      |      |
| +5D8   | +7D10                                                                   | 0                                            | +6D8  | 0      | +8D11 | 0      |      |
| +5D8   | +9D11                                                                   | 0                                            | +7D9  | 0      | +8D12 | 0      |      |
| 0D2    | 0D5                                                                     | 0                                            | +9D12 | 0D7    | 0D3   | 0D10   |      |
| 0D3    |                                                                         |                                              |       |        |       |        |      |

<sup>1</sup> Mice were injected with 15% India ink, 0.5 cc. intraperitoneally and 0.5 cc. intravenously, 8 and 26 hours following splenectomy, and the sensitized trypanosomes were given 4 hours after the last ink injection.

+ = trypanosome infection and the number after it shows the day after injection on which the trypanosomes were first seen; D = died and the number after it indicates the day after injection on which death occurred; 0 = no trypanosome infection.

quired, however, to prevent infection in splenectomized and blockaded mice given a similar number of trypanosomes. Thus, in the typical experiment shown in fig. 1 and table 1, experiment 1, at least 1.8 cc. was required to prevent infection of mice which had been splenectomized and blockaded with India ink, whereas 0.4 cc. protected normal mice. Since the blockaded mice required much more serum to prevent infection than normal mice, the protective titer of the serum was reduced in the blockaded mice.

This conclusion is corroborated by data from 5 other similar experiments in which the same or other material was used for the blockade and in which the same standard dose of trypanosomes was given. In these, at least 1.0, 1.4, 1.6, 1.5, 1.4 cc., respectively, were required to protect the splenectomized mice blockaded with India ink, normal sheep serum or normal dog serum, as compared to 0.4, 0.5, 0.5, 0.5, or 0.4 cc., respectively, necessary to protect normal mice (table 1, expers. 2-6). On the other hand, less of the immune serum (less than 0.3 cc.) was required to protect mice which had been splenectomized and blockaded with immune serum in contradistinction to



those blockaded with normal sheep or dog serum or India ink than was required for normal mice (0.6 cc.: see table 1, exper. 7). This experiment indicated that the immune serum was present in high concentration, not that blockaded failed to affect the mice.

When fewer trypanosomes were injected, smaller doses of the immune serum protected both normal and splenectomized-blockaded mice, and the effect of splenectomy plus blockade was less pronounced. Thus, when 1,200 to 1,600 trypanosomes were injected, 0.3 and 0.25 cc. sufficed to protect normals and only 0.5 cc. was required to protect splenectomized and blockaded mice (table 1, exers. 8 and 9).

Loss of the spleen is associated with the removal of considerable macrophage and macrophage-forming tissue,<sup>4</sup> especially in rats and mice whose ratio of spleen weight to body weight is higher than in many other animals.<sup>5</sup> From the results presented above, therefore, splenectomized mice might be expected, even without blockade, to require more immune serum than normal mice for protection against *T. equiperdum*. The results shown in fig. 1 and table 1, experiment 1, might indicate that splenectomy (performed 7 days before the protection test) lowers the protective titer of the immune serum, since the splenectomized mouse receiving 0.5 cc. of serum became infected whereas 0.4 cc. protected normal mice. Subsequent experiments (table 1, exers. 2-8) failed, however, to indicate that splenectomy alone definitely affects the protective titer of the immune serum, for 0.3, 0.5, 0.5, 0.5, 0.6, 0.5 and 0.3 cc., respectively, were required to protect the splenectomized mice, as compared to 0.4, 0.5, 0.5, 0.5, 0.4, 0.6 and 0.3 cc., respectively, necessary to protect normal mice. In these experiments the spleens were removed 7, 1, 1, 5/24, 1, 7 and 5/24 days before the protection tests, respectively.

#### THE RESISTANCE OF NORMAL AND OF SPLENECTOMIZED AND BLOCKADED MICE TO SENSITIZED TRYPANOSOMES

The following appeared to be the most likely explanations for the effect of splenectomy plus blockade upon the protective titer of the anti-trypanosome serum: (1) that splenectomy blockade inhibits phagocytosis of sensitized trypanosomes, (2) that it affects complement, thereby inhibiting trypanolysis, or (3) that it interferes with the specific sensitization of the trypanosomes *in vivo*. If splenectomized-blockaded mice should be less resistant to sensitized trypanosomes than normal mice, the first 2 explanations would be favored, but if they were as resistant as normal mice to sensitized trypanosomes the third interpretation would be supported. Normal and splenectomized-blockaded mice were injected, therefore, with equal numbers of trypanosomes which had been specifically sensitized *in vitro* (tables 2 and 3). In experiments 10 and 11 (table 2) they received subcutaneously the indicated amounts of a suspension containing 2,000 sensitized trypanosomes per 0.1 cc. In experiments 12-16 (tables 2 and 3) the suspension contained 1,000 sensitized trypanosomes per 0.1 cc. and was given intravenously. For the mice receiving 0.025 and 0.01 cc. of trypanosomes (exers. 11 and 12), 0.25 and 0.1 cc. of a 1:10

4. Taliaferro, W. H., and Mulligan, H. W.: Indian Medical Research Memoir 29, 1937.

5. Krumbhaar, E. R., and Musser, T. H., Arch. Int. Med. 31: 686, 1923.

dilution was given to insure accuracy. No effect of splenectomy and blockade was noted in experiments 10-12 (table 2), i.e., normal mice became infected as soon after injection and as frequently as did splenectomized and blockaded mice.

In the meantime, subsidiary experiments on the effect of blockade upon the retention of bromsulphalein from the blood stream indicated that fewer but more concentrated blockading doses of India ink than those previously employed probably caused greater macrophage injury since they prolonged the retention of the dye in the peripheral blood. Hence, mice in experiments 13-16 (table 3) were injected with 15% India ink (0.5 cc. intraperitoneally and 0.5 cc. intravenously) 8 and 26 hours after splenectomy, and the sensitized trypanosomes were given intravenously 4 hours after the last India ink injection. In experiment 13 (table 3) 5 splenectomized and blockaded and 5 normal mice were used and 3 of the former showed trypanosomes on the fourth, fifth and fifth days after injection. The remaining 2 died on the second and third days before evidence of infection was noted. On the other hand, 3 of the normal mice failed to become infected and trypanosomes were not seen in the blood of the other 2 until the eighth and eleventh days after injection. This experiment appeared to indicate that blockade reduces the resistance of mice to specifically sensitized trypanosomes. Three later experiments, however, each employing 8 normal and 8 splenectomized and blockaded mice, failed to reveal any difference between the susceptibility of normal and of blockaded mice (experiments 14-16, table 3).

An explanation for these divergent results cannot be given, but the following observation suggests that the effect observed in experiment 13 might be due to the moribund condition of the mice. At the time the sensitized trypanosomes were injected, the blockaded mice in experiment 13 were apparently moribund. When tail blood from these mice was streaked on blood agar, numerous colonies of a gram negative bacterium were obtained in pure culture. These organisms were found in the India ink which had been injected. Blood from the blockaded mice of the following two experiments also contained large numbers of the same bacterium. These mice, although ill in appearance, were not affected to the extent observed in the first case. In the final experiment (16, table 3) the ink, before injection, was purposely inoculated with a heavy suspension of the organism. Again, however, blockade failed to lower resistance to the sensitized trypanosomes.

#### DISCUSSION

The present study shows that splenectomized and blockaded mice require more immune sheep serum than normal or splenectomized mice for protection against *T. equiperdum*, i.e., the passive protective titer of the immune sheep serum is reduced by splenectomizing and blockading the recipient mice.

Direct interpretation of these findings is difficult, for definite proof of macrophage blockade is lacking. Several lines of evidence tend to indicate, however, that the so-called blockading technic affects the macrophages to a considerable degree, especially when combined with splenectomy. In the first place, splenectomy removes many functional macrophages and cells which



can develop into macrophages.<sup>4</sup> In addition, macrophages remove injected colloids from the circulation and it is reasonable to suppose that continued phagocytosis of such particles would eventually retard the activity of the cells. A state of impaired activity would appear to explain the induction of relapses in malaria infection in birds by injecting large quantities of foreign erythrocytes (Gingrich<sup>6</sup>), since Taliaferro and Cannon<sup>7</sup> and Taliaferro and Mulligan<sup>4</sup> showed that the macrophages play the chief role in malarial immunity. Finally, the blockade employed in the present investigation profoundly retards the rate of removal of bromsulphalein from the peripheral blood of mice (unpublished experiments). Mills and Dragstedt<sup>8</sup> recently published observations indicating that the bromsulphalein retention test is a measure of the functional activity of the "reticulo-endothelial" system.

Assuming that blockade does greatly reduce the functional efficiency of the macrophages and that the effect of splenectomy blockade noted in the present investigation may be explained on this basis, it is still difficult to visualize the exact mechanism responsible for the reduced protective titer of the immune serum. Interference with phagocytosis, decreased complement<sup>9</sup> or reduced opsonin<sup>10</sup> cannot be held responsible, because splenectomy-blockade does not reduce resistance to sensitized trypanosomes, nor does it appear to affect the rate of disappearance of sensitized trypanosomes from the blood.<sup>2</sup> Blockade seems, therefore, to interfere with the specific sensitization of trypanosomes in vivo, perhaps, as Bieling<sup>11</sup> and Taliaferro<sup>12</sup> suggested, by interfering with the concentration of passively acquired antibodies in macrophage tissues. Experiments by the author<sup>13</sup> support this explanation, for it was found that passively acquired antibodies are definitely retained for longer periods in the peripheral blood of blockaded mice.

Complicating to any explanation of the effect of splenectomy and blockade upon the protective titer of the immune serum was the incidental finding by the author<sup>13</sup> that unilateral nephrectomy or uretectomy is accompanied by a slight reduction in the protective titer of the sheep anti-T. equiperdum serum. Taliaferro<sup>2</sup> could not corroborate this finding in rats injected with rat anti-T. lewisi serum.

#### CONCLUSIONS

The protective titer of sheep anti-T. equiperdum serum, when injected intraperitoneally in mice, is appreciably reduced provided the mice are splenectomized and blockaded with India ink or normal serum and injected subcutaneously with 2,500 to 3,000 trypanosomes (fig. 1 and table 1, expts. 1-6), but is not reduced if the mice are only splenectomized (fig. 1 and table 1, expts. 1-8) or if the mice are splenectomized and blockaded

6. J. Parasitol. **20**: 332, 1934.

7. J. Prev. Med. **5**: 37, 1931; J. Infect. Dis. **59**: 72, 1936.

8. Proc. Soc. Exp. Biol. & Med. **34**: 228, 1936.

9. Jungeblut, C. W., and Berlot, J. A.: J. Exper. Med. **43**: 797, 1926.

10. Elvidge, A. R.: J. Immunol. **24**: 31, 1933.

11. Centralbl. f. Bakt. (Abt. 1) O. **110**: 195, 1929.

12. J. Parasitol. **20**: 149, 1934.

13. Dissertation, Univ. of Chicago. 1936.

with specific (i.e., sheep anti-*T. equiperdum*) serum (table 1, exper. 7). Furthermore, it is only slightly reduced in mice splenectomized and blocked with India ink and injected subcutaneously with 1,200 to 1,600 trypanosomes (table 1, expts. 8 and 9). Splenectomy plus blockade appears to have no effect upon the resistance of mice to trypanosomes specifically sensitized in vitro (tables 2 and 3). The effect of splenectomy and blockade upon the protective titer of trypanocidal serum cannot, therefore, be due to inhibited phagocytosis, decreased complement or reduced opsonin, but it may be due to interference with the concentration of passively acquired antibodies in macrophage tissues.













